

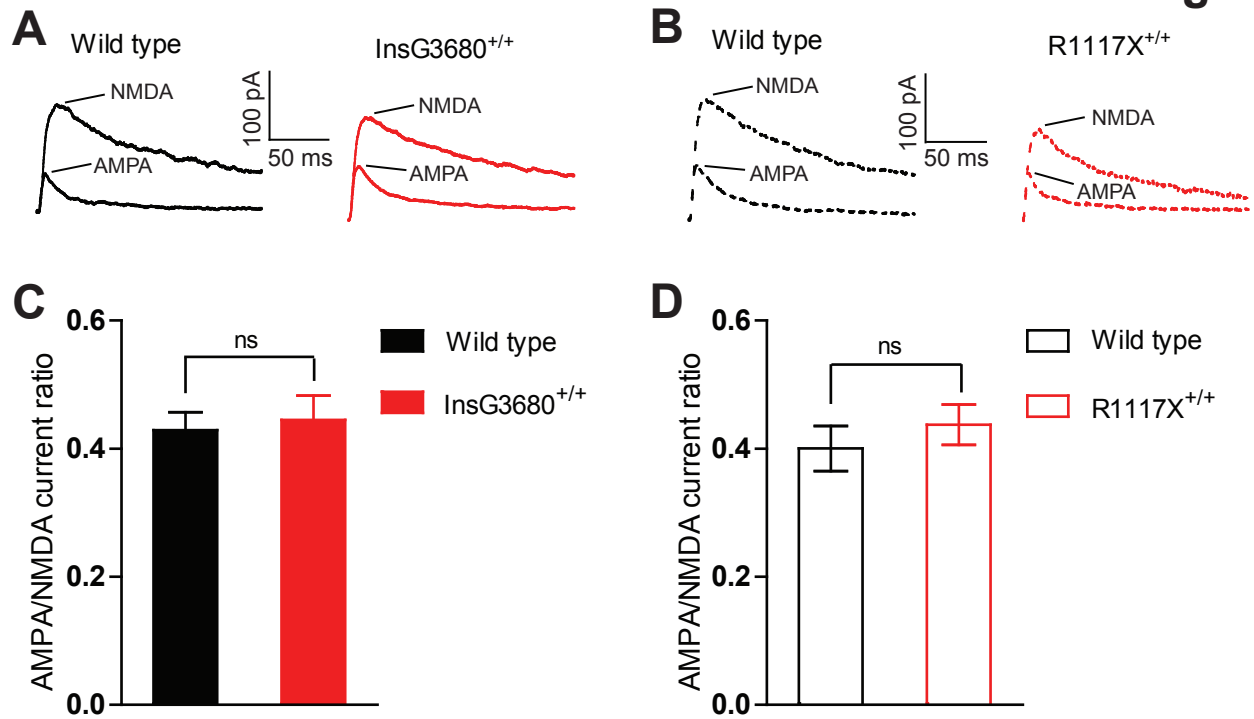
Fig S1

Fig S1. No change of AMPA to NMDA ratio in MSNs from $R1117X^{+/+}$ and $InsG3680^{+/+}$ at P14. Related to Figure 2.

(A and B) Representative AMPA current traces and digitally subtracted NMDA current traces from striatal MSNs with indicated genotypes.

(C and D) No significant difference of AMPA to NMDA current ratio between $InsG3680^{+/+}$, $R1117X^{+/+}$ mice and their wild type littermates. In $InsG3680$ cohort, $n = 14$ neurons for wild type, $n = 16$ neurons for $InsG3680^{+/+}$ from three pairs of littermates; in $R1117X$ cohort, $n = 18$ neurons for wild type, $n = 19$ neurons for $R1117X^{+/+}$ from three pairs of littermates. Data are presented as mean \pm SEM; two-tailed student t-test.

Fig S2

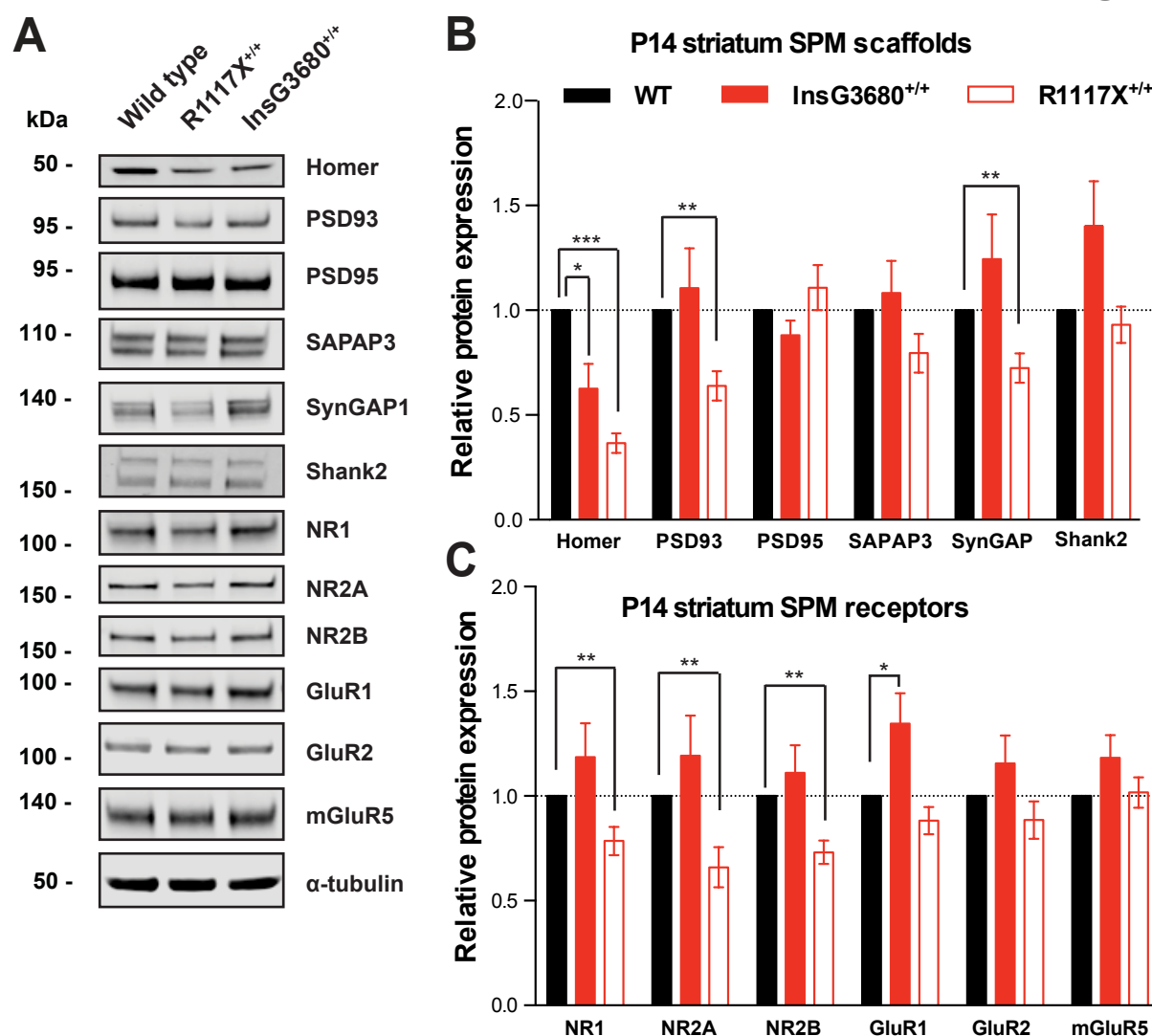


Fig S2. R1117X^{+/+} and InsG3680^{+/+} mice show differential abnormalities in striatal protein expression during early development (p14). Related to Figure 2.

(A) Representative blots for proteins detected by specific antibodies in the striatal SPM fraction from wild type, InsG3680^{+/+} and R1117X^{+/+} mice.

(B and C) Quantification of relative levels of proteins as normalized to tubulin expression from striatal SPM. (n = 8 samples per protein per genotype, each n being pooled tissue from three mice).

Data are presented as mean ± SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; one sample t-test.

Fig S3

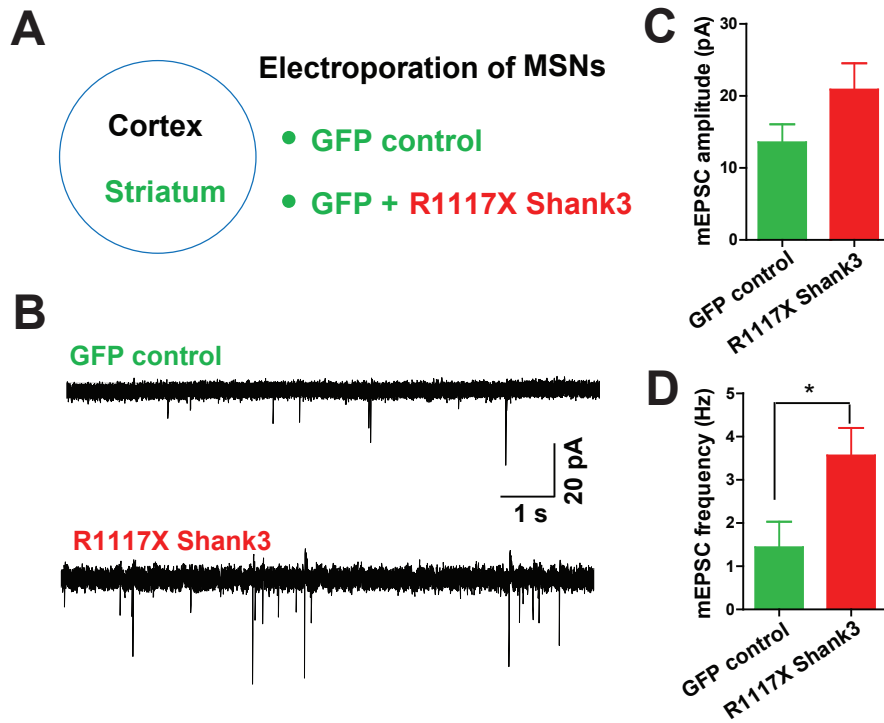


Fig S3. R1117X Shank3 increases synaptic transmission in young MSNs.

Related to Figure 2.

(A) Schematic diagram of experimental design. Striatal MSNs derived from P0 Shank3B knock mice were electroporated with either GFP plasmid or GFP plus R1117X Shanks plasmids, then plated with cortical neuron at a ratio of 3:1

(B) Representative mEPSC traces from young MSNs at DIV 8 transfected with indicated plasmids.

(C and D) R1117X Shank3 transfected MSNs tend to display increased mEPSC amplitude and significantly higher mEPSC frequency at DIV 8. Data are presented as mean \pm SEM. * $P < 0.05$; two tail t-test. $n = 5$ neurons per group.

Fig S4

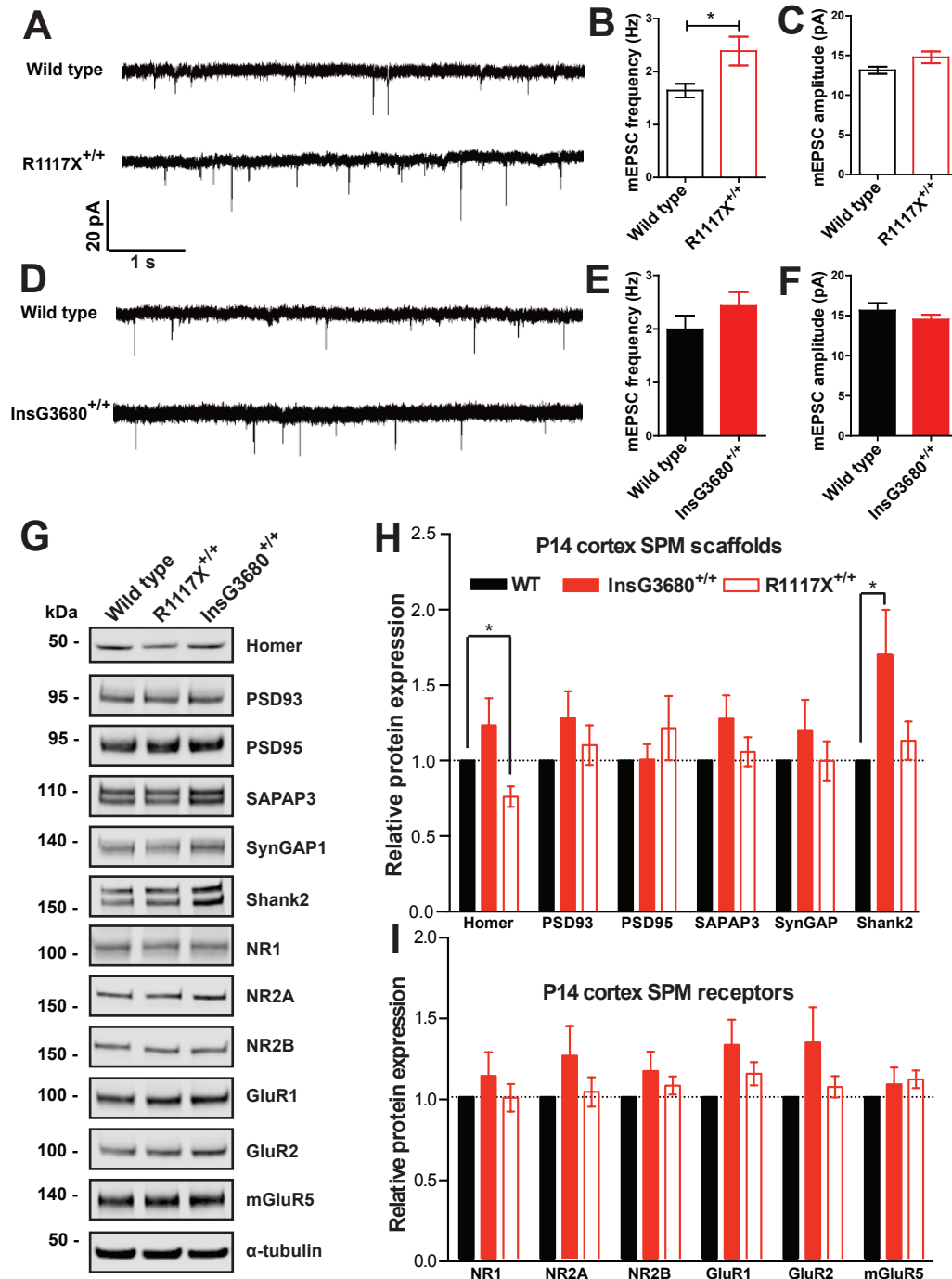


Fig S4. Electrophysiological, biochemical characterization of cortical synaptic transmission and protein expression during early development (p14). Related to Figure 2. (A-C) mPFC neurons of R1117X^{+/+} mice display increased frequency of mEPSCs at P14. $n = 21$ neurons for wild type; $n = 18$ neurons for R1117X^{+/+} from three pairs of littermates. (D-F) mPFC neurons of InsG3680^{+/+} mice display normal frequency and amplitude of mEPSCs at P14. $n = 20$ neurons for wild type; $n = 19$ neurons for InsG3680^{+/+} from three pairs of littermates. Data are presented as mean \pm SEM. * $P < 0.05$; two-tailed t-test. (G) Representative blots for proteins detected by specific antibodies in cortical SPM fraction. (H and I) Quantification of relative levels of proteins as normalized to tubulin expression from cortical SPM. ($n = 8$ samples per protein per genotype, each n being pooled tissue from two mice). Data are presented as mean \pm SEM. * $P < 0.05$; one sample t-test.

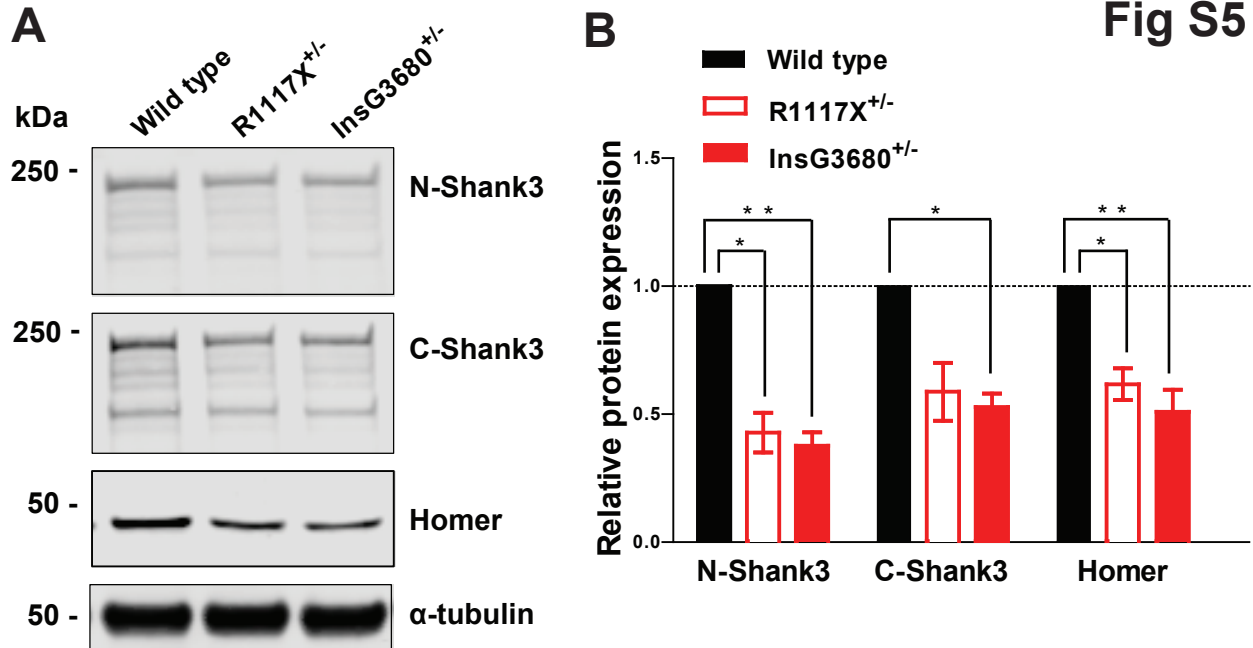


Fig S5. Reduced Shank3 and Homer protein expression in both R1117X and InsG3680 heterozygous mice. Related to Figure 6.

(A) Representative western blots using striatal SPM prepared from wild type, R1117X^{+/-} and InsG3680^{+/-} mice with indicated antibodies.

(B) Quantification of relative levels of proteins as normalized to tubulin protein expression from striatal SPM. ($n = 4$ samples per protein per genotype, each n being pooled tissue from three mice). Data are presented as mean \pm SEM. * $P < 0.05$, ** $P < 0.01$; one sample t-test.

Fig S6

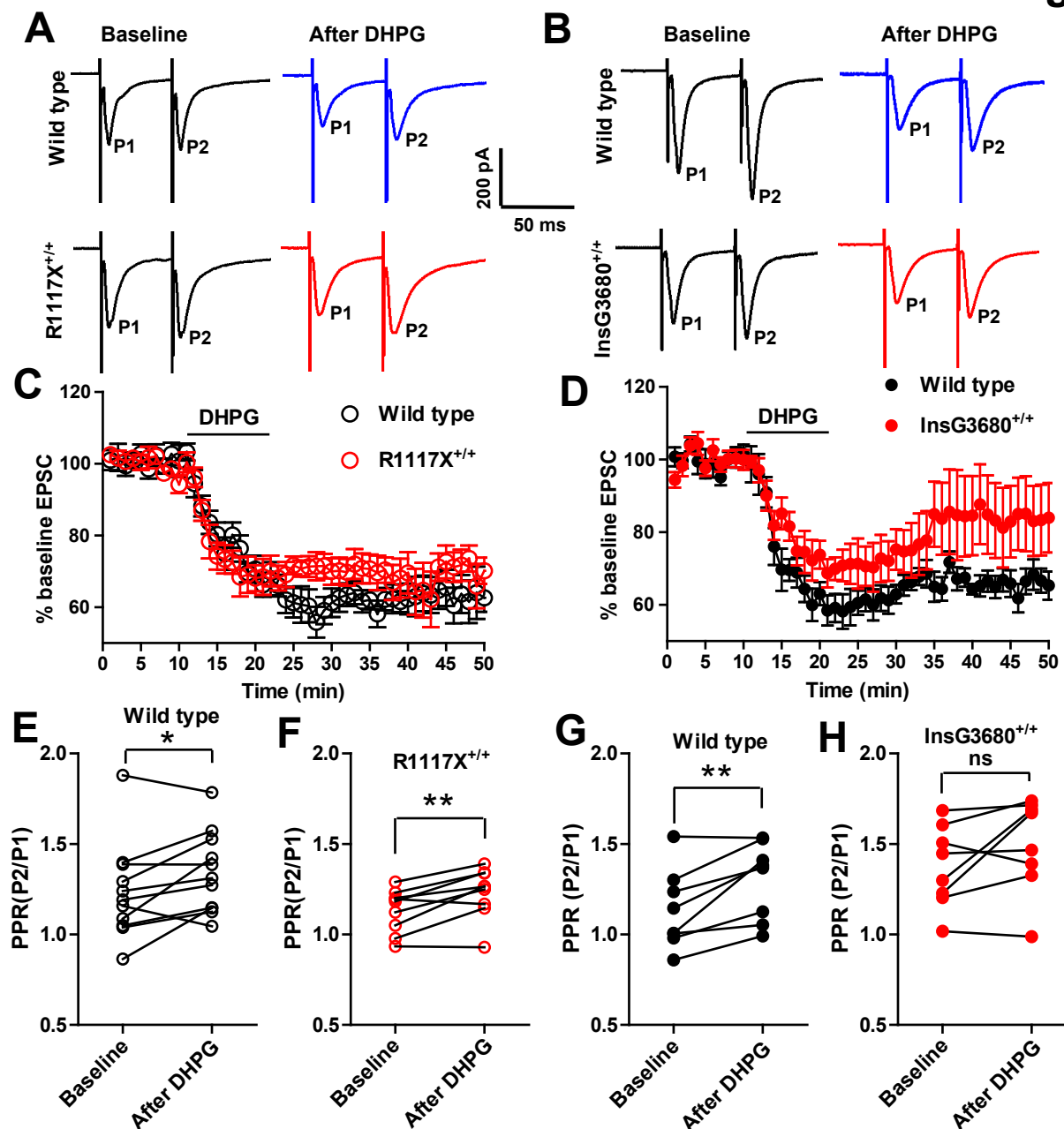


Fig S6. DHPG induced striatal LTD mediated by group I mGluRs is impaired in InsG3680^{+/+} but not R1117X^{+/+} mice. Related to Figure 6.

(A and B) Representative evoked EPSC traces (averaged amplitude of responses during the 10 min baseline and 15-25 min after DHPG perfusion) from neurons with indicated genotypes.

(C and D) Normalized amplitude of first peak (P1) of evoked EPSCs as a function of time before, during and after DHPG perfusion. In the R1117X group, $n = 11$ neurons for wild type, $n = 9$ neurons for R1117X^{+/+} from 4 pairs of mice. In the InsG3680 group, $n = 8$ neurons for wild type, $n = 8$ neurons for InsG3680^{+/+} from 4 pairs of mice.

(E-H) Increased paired pulse ratio after DHPG perfusion in wild types from both lines and R1117X^{+/+} but not InsG3680^{+/+} mice. Data are presented as mean \pm SEM, * $P < 0.05$, ** $P < 0.01$; paired two-tailed t-test.

Fig S7

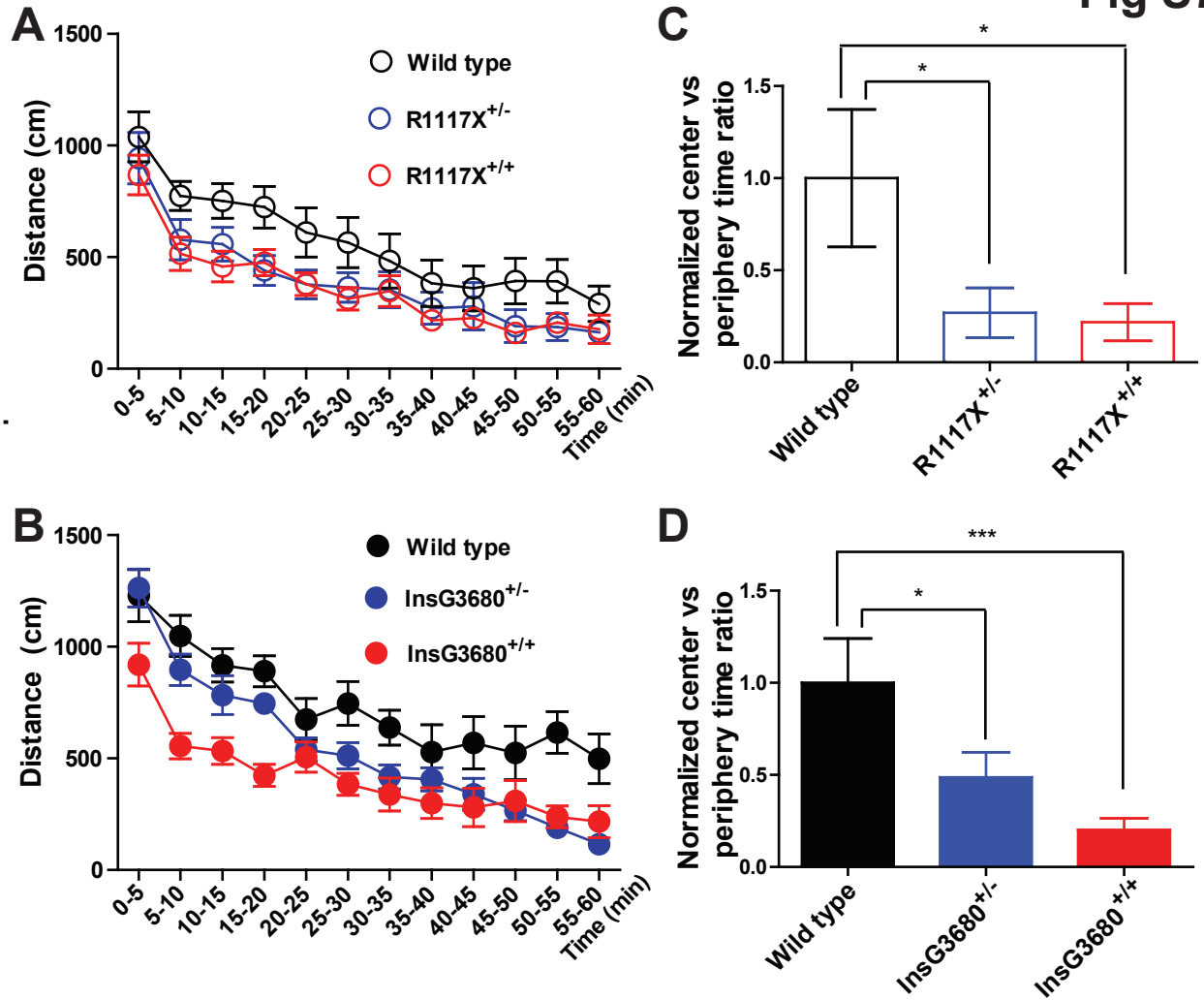


Fig S7. Normal habituation and reduced center vs periphery time ratio during open field test in both R1117X and InsG3680 Shank3 mutant mice. Related to Figure 7.

(A and B) Distance travelled as plotted every 5 min during open field test in R1117X and InsG3680 cohorts. In the R1117X cohort, $n = 15$ mice for wild type; $n = 15$ mice for R1117X^{+/-}; $n = 15$ mice for R1117X^{+/+}. In the InsG3680 cohort, $n = 17$ mice for wild type; $n = 19$ mice for InsG3680^{+/-}; $n = 18$ mice for InsG3680^{+/+}. Data are presented as mean \pm SEM. Significant differences of distance ($*** P < 0.001$) were found from all genotypes using time as variable factor with two-way ANOVA test.

(C and D) Center vs periphery time ratio from R1117X and InsG3680 cohorts in the open field test as normalized to their wild type littermates. Data are presented as mean \pm SEM, $* P < 0.05$, $*** P < 0.001$; one-way ANOVA with Bonferroni post hoc test.

Fig S8

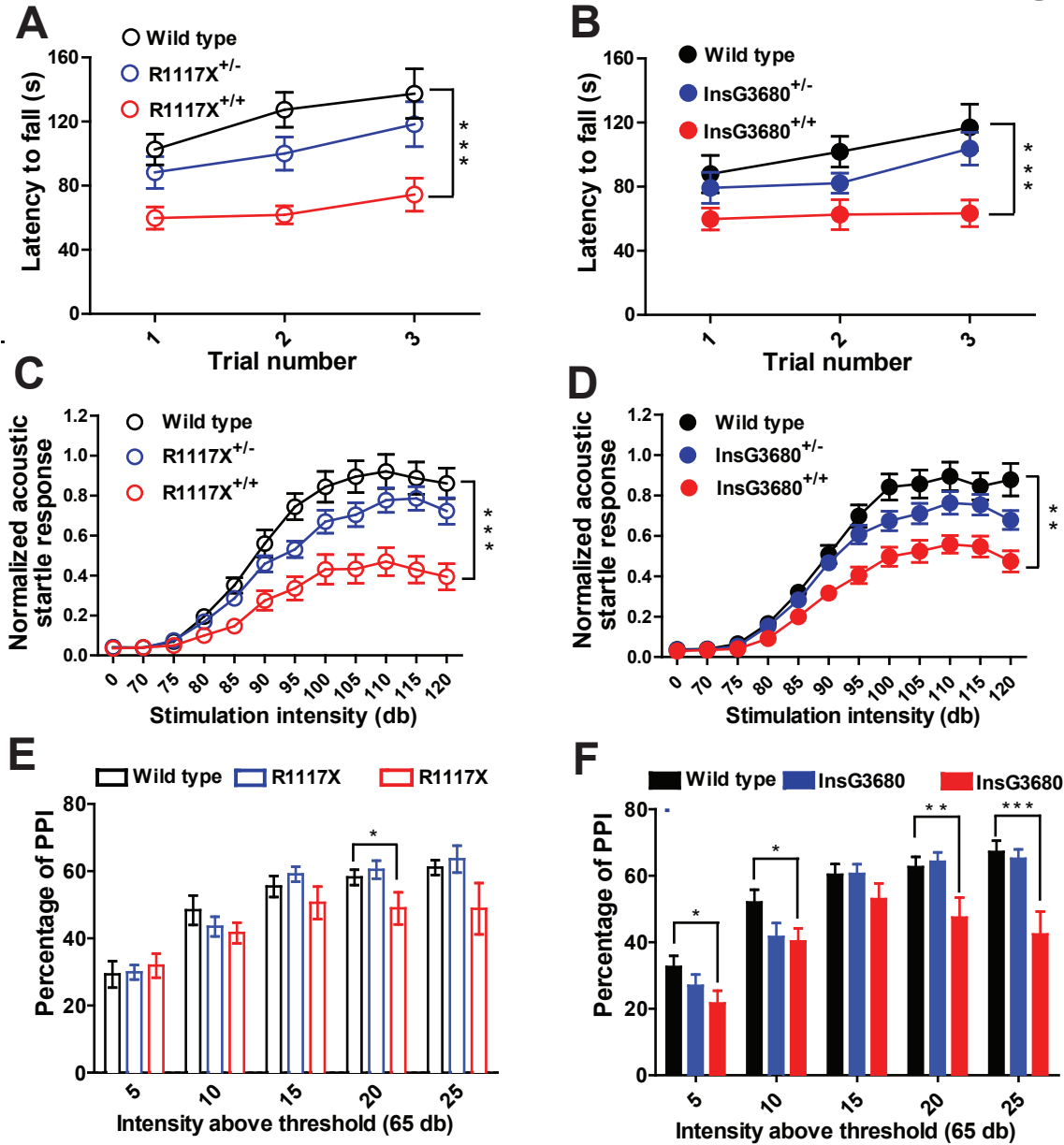


Fig S8. Impaired motor learning and startle response in both R1117X^{+/+} and InsG3680^{+/+} mice. Related to Figure 8.

(A and B) Statistical results of rotarod test from R1117X and InsG3680 cohorts. In the R1117X cohort, $n = 15$ mice for wild type; $n = 15$ mice for R1117X^{+/-}; $n = 15$ mice for R1117X^{+/+}. In the InsG3680 cohort, $n = 16$ mice for wild type; $n = 19$ mice for InsG3680^{+/-}; $n = 18$ mice for InsG3680^{+/+}. Data are presented as mean \pm SEM, *** $P < 0.001$; two-way ANOVA with Bonferroni post hoc test.

(C and D) Impaired acoustic startle response in both R1117X^{+/+} and InsG3680^{+/+} mice.

(E and F) Impaired pre-pulse inhibition in both R1117X^{+/+} and InsG3680^{+/+} mice. In R1117X cohort, $n = 16$ mice for wild type; $n = 18$ mice for R1117X^{+/-}; $n = 16$ mice for R1117X^{+/+}. In InsG3680 cohort, $n = 16$ mice for wild type; $n = 16$ mice for InsG3680^{+/-}; $n = 14$ mice for InsG3680^{+/+}. Data are presented as mean \pm SEM, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; two-way ANOVA with Bonferroni post hoc test.

Supplemental table S1 (Related to Figures 1, 5, 6):

List of antibodies and their working condition

Protein	Antibody	Species	Vendor	Dilution	Blocking Buffer
SynGAP	D88G1	Rabbit mAb	CST	1 to 1000	5% BSA
GluR1	#MAB2263	Mouse	Millipore	1 to 1000	5% milk
GluR2	32-0300	Mouse (IgG2a)	Invitrogen	1 to 500	5% milk
PSD95	ab18258	Rabbit	Abcam	1 to 1000	5% milk
Homer	AB5875	Rat	Chemicon	1 to 1000	5% milk
NR2A	07-632	Rabbit	Millipore	1 to 1000	5% milk
NR2B	N59/36	Mouse	NeuroMAB	1 to 1000	5% milk
NR1	556308	Mouse (IgG2a)	BD Pharmingen	1 to 1000	5% milk
Shank1	162 002	Rabbit	Synaptic Systems	1 to 1000	5% BSA
Shank2	#12218	Rabbit	CST	1 to 500	5% BSA
Shank3N	N367/62	Mouse (IgG2a)	NeuroMab	1 to 100	5% milk
Shank3C	SC-30193	Rabbit	Santa Cruz	1 to 1000	5% milk
SAPAP3	homemade	Rabbit	Feng lab	1 to 1000	5% milk
PSD93	ABR-01252	Rabbit	Dianova	1 to 1000	Odyssey blocking buffer
mGluR5	ab76316	Rabbit	Abcam	1 to 5000 (unboiled sample)	5% milk

Supplemental Table S2. Molecular, biochemical, morphological, electrophysiological and behavioral phenotypes of R1117X and InsG3680 Shank3 mutant mice. Related to Figures 1-8.

		R1117X Shank3		InsG3680 Shank3	
		Heterozygous	Homozygous	Heterozygous	Homozygous
Molecular changes of Shanks	Shank3 mRNA	ND	reduced	ND	reduced
	Shank1 mRNA	ND	normal	ND	Upregulated in cortex
	Shank2 mRNA	ND	normal	ND	Upregulated in cortex
Biochemical changes of Shanks	Shank3 protein	About 50% reduced	Expression of truncated R1117X Shank3 isoform	About 50% reduced	No expression of truncated InsG3680 Shank3 isoform detected
	Shank1 protein	ND	normal	ND	Trend of increase in cortex
	Shank2 protein	ND	normal	ND	Upregulated in cortex
Biochemical changes of other synaptic proteins	Adult cortical SPM	Reduced Homer	Reduced Homer, PSD95, PSD93, SynGap, NR1	Reduced Homer	Reduced Homer, PSD95, PSD93, Trend of reduced NR1 and SynGap
	P14 cortical SPM		Reduced Homer, trend of increased GluR1		Trend of increased scaffolding proteins and receptors
	Adult striatal SPM	Reduced Homer	Reduced Homer, PSD95, SynGap, NR1, NR2B, Trend of reduced NR2B, SAPAP3 and GluR2	Reduced Homer	Reduced Homer, SAPAP3, SynGap, NR1, NR2A, NR2B, GluR2, mGluR5
	P14 striatal SPM	ND	Reduced Homer, PSD93, SynGap and NMDA receptor subunits	ND	Reduced Homer; Trend of increased scaffolding proteins and receptors; Significant increase of GluR1
Synaptic and electrophysiological phenotypes	Adult Prefrontal cortex	Reduced spine number; Reduced mEPSC frequency	Reduced spine number; Reduced mEPSC frequency and amplitude	No reduction detected	Reduced spine number
	P14 prefrontal cortex	ND	Increased mEPSC frequency	ND	Normal

	Adult dorsal lateral striatum	Reduced mEPSC amplitude	Reduced mEPSC frequency and amplitude; Reduced pop spike amplitude; Reduced NMDA current; normal DHPG induced LTD	Reduced mEPSC frequency	Reduced mEPSC frequency and amplitude; Reduced pop spike amplitude; Reduced NMDA current; impaired DHPG induced LTD
	P14 dorsal lateral striatum	ND	Normal	ND	Reduced pop spike amplitude; Increased mEPSC amplitude
Behavioral phenotypes	Pups ultrasonic vocalization	ND	Normal	ND	Normal
	Juvenile social play	ND	normal	ND	Reduced reciprocal interaction
	Anxiety related behaviors	Increased anxiety level	Increased anxiety level	Increased anxiety level	Increased anxiety level
	Locomotor activity	Reduced	Reduced	Reduced	Reduced
	Working memory tested by T maze	ND	Normal	ND	Normal
	Acoustic startle response	Normal	Impaired	Normal	Impaired
	Prepulse inhibition test	Normal	Mild impaired	Normal	Severely impaired
	Motor learning and coordination	Normal	Impaired	Normal	Impaired
	Social interaction	Impaired	Impaired	Normal	Impaired
	Repetitive behaviors	Normal	Trend of increase repetitive grooming; Low penetrance of skin lesion	Normal	Increased repetitive grooming; High penetrance of skin lesion
	Allogrooming and dominant behavior	High penetrance of allogrooming; Increased dominance-like behavior	No allogrooming behavior; Increased dominance-like behavior	Low penetrance of allogrooming; No dominance-like behavior	No allogrooming behavior; Increased dominance-like behavior

Supplemental Experimental Procedures

Animal work statement

All animal related work was performed under the guidelines of Division of Comparative Medicine (DCM), with protocol (# 0513-044-16 of Feng laboratory and #1012-102-15 of Lewis laboratory) approved by Committee for Animal Care (CAC) of Massachusetts Institute of Technology, and was consistent with the Guide for Care and Use of Laboratory Animals, National Research Council 1996 (institutional animal welfare assurance no. A-3125-01). Only aged-matched male mice were used for all behavioral experiments, all other tests included age-matched males and females in proportional contribution across groups.

Generation and breeding of mutant mice

R1117X and InsG3680 *Shank3* mutant mice were generated by homologous recombination in R1 mouse ES cells and implanted in C57 blastocysts using standard procedures. R1117X and InsG3680 *Shank3* targeting vectors were constructed and introduced separately into R1 ES cells to replace the corresponding wild type allele. Homology arms with about 800 bp of size on both sides were designed to flank the desired point mutation, a pair of TALE nuclease plasmids were also designed as previously described (Sanjana et al., 2012) to facilitate the recombination efficiency through co-electroporation into ES cells together with targeting vector. The neo cassette was removed after targeting. High percentage chimeric mice were backcrossed with C57BL/6J (Jackson laboratory) to establish germline transmission followed by backcrossing with C57 BL/6J for five to six generations. Genotypes were determined by PCR of mouse tail DNA using the

following primer pairs and gave rise to a band of 400 base pairs for wild type allele and 478 for R1117X mutation allele:

Primer F (5'-ATCTGCCATCCCTACAACCCTCC-3');

Primer R (5'-TCTCTCCGAGCAGGCACTGGAATCC-3')

For InsG3680, the following primer pairs were applied and generated a band of 407 base pairs for wild type allele and 486 for InsG3680 mutation:

Primer F (5'-TGCAAACCCGAGACTCTGAGAGAGG -3');

Primer R (5'-AGCGAATACCAGCTCTGGCTCCTCC -3').

To build the colony with C57 B6/S129 Sv mixed genetic background, we crossed the F5 heterozygous mutants with S129 Sv wild type mice (Jackson laboratory) to generate the founder population, and then set up heterozygous x heterozygous brother-sister mating between these founders to generate offspring with C57B6/S129Sv mixed genetic background for all the experiments in this study. Animals were housed at a constant temperature of 23°C with a 12 h light/dark cycle with free access to food and water. Mice were housed 4–5 by genotype per cage with the exception of the animals individually housed for grooming assay.

Behavioral studies

All behavioral studies were carried out and analyzed with experimenter blinded to genotype. For all assays, mice were habituated in the test facility for 1 hour prior to starting the task. Each cohort of mice were used for maximally three behavioral tests with at least five days' break between tasks.

Grooming behavior test

Adult male mice about 5 month-old were used for analysis of grooming behavior. Animals were individually housed for 2 weeks before video tapping for 2 hours under red light illumination between 7:00-9:00 pm. Grooming behaviors were manually coded and analyzed using Noldus Observer software, so that the total duration that an animal spent on grooming in the 2 hours segment was determined and normalized to its mobile time. Grooming included all sequences of face-wiping, scratching/rubbing of head and ears, and full-body grooming.

Open field

Spontaneous locomotor activity was assessed as total distance travelled (m) over 60 min in an automated Omnitech Digiscan apparatus (AccuScan Instruments) as described previously (Peca et al., 2011). Activity was quantified over a 60 minute period by a computer operated detecting system.

Elevated zero maze test

The elevated zero maze consists of a circular platform that is equally divided into four quadrants. The zero maze was indirectly illuminated at 60 lux on the open arms and 10 lux on the closed arms. Testing started with an animal being placed into a closed arm of the maze. Behavior was video-taped for 5 min and then scored by a trained observer using Noldus Observer software.

Anxiety-like behavior was interpreted based upon the percentage of time that mice spent in the open arms. The animals used in the elevated zero maze test were previously tested in the open field assay with one-week's break.

Three-chamber social test

Sociability and social novelty test were performed as previously described (Chao et al., 2010; Peca et al., 2011) with minor modifications. Briefly, 3 months-old male mice were used across all cohorts. Both stranger 1 and stranger 2 were wild type male S129 Sv males (Jackson laboratory) with matched age and body weight to test mice. Stranger mice were habituated by placing them inside an inverted wire cup for 30 min, two sessions per day for three consecutive days before experiments. Each stranger mouse was used maximally two times per day. Test mice were habituated to the facility environment for 1 hour before the start of behavioral tasks. The social test apparatus was made of a clear plexiglass box (65 (L) × 44 (W) × 30 (H) cm) with removable floor and partitions dividing the box into left, center, and right chambers. Center chamber (21 cm x 22 cm) is half the width of left (21 cm x 44 cm) and right chamber (21 cm x 44 cm). These three chambers were interconnected with 5 cm openings between each chamber which can be closed or opened manually with a lever operated door. The inverted wire cups to contain the stranger mice were cylindrical, 10 cm in height, a bottom diameter of 10 cm with the metal bars spaced 0.8 cm apart. A weighted cup was placed on top of the inverted wire cups to prevent the test mice from climbing onto the wire cup. Each wire cup was used only one time per day then followed by extensive clean with 75% ethanol and water at the end of the test day. During the habituation phase, an empty wire cup was placed into left and right chamber, and the test mouse was placed into the middle chamber and allowed to explore for 15 minutes, with the doors into both side chambers

open. During the sociability test phase, the test mouse was firstly gently introduced to the middle chamber with the doors to both side chambers closed, and an unfamiliar mouse (S1) was placed under the inverted wire cup in one of the side-chambers and a toy object (O) was placed under the inverted wire cup placed on the opposite side chamber. The location of the stranger mouse and object was counterbalanced between test trials to exclude side preference. The experimenter then lifted up the doorways to both side chambers simultaneously, and the test mouse was allowed to explore all three chambers for 15 minutes. During the social novelty test phase, the test mouse was again gently introduced into the middle chamber with the doors to both side chambers closed. Then a novel mouse (S2) was placed under the inverted wire cup, replacing the toy object (O) in one of the side-chambers. The experimenter then lifted up the doorways to both side chambers simultaneously, and the test mouse was allowed to explore all three chambers for an additional 15 minutes. Time spent in close proximity to the stranger mice or toy object was analyzed using the Noldus Ethovision software. The animals used in the social interaction test were previously tested in the zero maze assay with one-week's break.

Tube test

The tube test assay was performed as previously described (Wang et al., 2011a). We used transparent plexiglass tubes with 30 cm length and 3 cm inner diameter. This narrow space is just sufficient for an adult mouse (4-5 month-old) to walk through without being able to reverse its body direction. Mice were habituated to walk through the tube two sessions per day for four consecutive days before testing. On the day of testing, two unfamiliar mice with different genotypes were firstly met in the middle of the tube then released at opposite ends of the plexiglass tube simultaneously. The mouse that completely retreated firstly from the tube within first 6 min

of the test was defined as the loser, and the other as the winner. In very rare cases, when no mice retreated within 6 min, the tests were repeated. The same pair of mice was matched again on the same test day with mice entry sides counterbalanced. The tubes were cleaned with 75% ethanol between trials. A chi-square analysis was applied to determine the significance of test score between mice when compared with an outcome expected by chance (i.e. a 50:50 win–lose outcome).

Electrophysiological recordings

All electrophysiological measurement were performed and analyzed with experimenter blinded to genotype.

Slice preparation for adult mice

Acute brain slices were prepared from 8-weeks-old mice as described previously (Peca et al., 2011). Slices were prepared from a pair of mice consists of one wild type, one heterozygous and one homozygous with the same solution and procedure each day. Mice were deeply anesthetized by intra-peritoneal injection of avertin solution (20 mg/ml, 0.5 mg/g body weight) and then transcardially perfused with 25 ml of carbogenated (95% O₂, 5%CO₂) ice cold cutting artificial cerebrospinal fluid (aCSF) with the composition (in mM): 105 NMDG, 105 HCl, 2.5 KCl, 1.2 NaH₂PO₄, 26 NaHCO₃, 25 Glucose, 10 MgSO₄, 0.5 CaCl₂, 1 L-Ascorbic Acid, 3 Sodium Pyruvate, 2 Thiourea (pH 7.4, with osmolarity of 300–310 mOsm). The brains were rapidly

removed and placed in ice-cold and oxygenated cutting solution. Coronal slices (300 μm) were sliced using Leica VT1200S (Leica Microsystems) and then transferred to recovery chamber at 32 °C with carbogenated cutting aCSF solution for 12 min, followed by transferring to holding chamber containing aCSF that contained (mM): 119 NaCl, 2.3 KCl, 1.0 NaH₂PO₄, 26 NaHCO₃, 11 Glucose, 1.3 MgSO₄, 2.5 CaCl₂ (pH was adjusted to 7.4 with HCl, with osmolarity of 300–310 mOsm) at room temperature. Slices were allowed to recover for one more hour in holding chamber before recording and used for experiment typically between 2~6 hours after slicing.

Slice preparation for juvenile mice

Acute brain slices from juvenile mice were prepared as described previously (Wan et al., 2011). Briefly, a pair of wild type and homozygous mice from the same litter at postnatal day 14 were deeply anesthetized and rapidly decapitated. Dissected brain were placed into carbogenated (95% O₂, 5%CO₂) ice cold cutting artificial cerebrospinal fluid (aCSF) with the composition (in mM) 194 sucrose, 30 NaCl, 4.5 KCl, 1.2 NaH₂PO₄, 26 NaHCO₃, 10 glucose, 0.2 CaCl₂, 8 MgSO₄ (pH 7.4, 350 mOsm). Coronal slices (300 μm) were sliced in the same cutting aCSF using Leica VT1200S (Leica Microsystems) and then transferred into a recovery chamber containing carbogenated normal aCSF of the composition (in mM): 119 NaCl, 2.3 KCl, 1 NaH₂PO₄, 26 NaHCO₃, 11 glucose, 2.5 CaCl₂, 1.3 MgSO₄ (pH 7.3, 300–310 mOsm) for 15 min at 32 °C before moving to the holding chamber at room temperature. Slices were allowed to recover for one more hour in holding chamber before recording and used for recording typically between 2~6 hours after preparation.

Cortico-striatal extracellular field recording

Recording of population spike in dorsal lateral striatum was performed as described (Peca et al., 2011; Welch et al., 2007). Slice was placed into recording chamber (Warner Instruments) and constantly perfused with oxygenated aCSF at room temperature at a speed of 2.0 ml/min. A platinum iridium concentric bipolar electrode (FHC) was positioned on the inner border of the corpus callosum between the cortex and dorsolateral striatum to stimulate the predominant cortical input to dorsolateral striatal region. A borosilicate glass recording electrodes filled with 2M NaCl was placed onto the dorsolateral striatal region approximately 400 μm away from the stimulating electrode. Cortico-striatal field population spikes were elicited by delivery step depolarization (0.15 ms duration with 0.5 mA intensity at a frequency of 0.1 Hz). Stable baseline response of pop spike for at least of 5 min from individual slice was ensured before moving to input-output assay. Input-output curves were determined for both the negative peak 1 (NP1; presynaptic fiber volley) and pop spike amplitude by delivery three consecutive stimulation from 0 to 1 mA with 0.1 mA increments. Recordings were performed at room temperature and data were sampled using pCLAMP 10 software (Molecular Devices).

Whole cell patch clamp recording

Slice was placed into recording chamber (Warner Instruments) and constantly perfused with oxygenated aCSF at room temperature at a speed of 2.0 ml/min. MSNs were visually identified with a microscope equipped with IR-DIC optics (BX-51WI, Olympus) by location, shape and other electrophysiological parameters. Recording pipettes with resistance about 3~4 M Ω were pulled with pipette puller (P-97, Sutter Instruments) using capillary glass (King Precision Glass,

type 8250). Pipettes were filled with internal solution containing (in mM): 107 CsMeSO₃, 10 CsCl, 3.7 NaCl, 5 TEA-Cl, 20 HEPES, 0.2 EGTA, 5 lidocaine N-ethyl chloride, 4 ATP magnesium salt, and 0.3 GTP sodium salt (pH 7.3, osmolarity 300~305 mOsm). Series resistance between 10 to 15 M Ω was constantly monitored. Multiclamp 700B amplifier (Molecular Devices) and digidata 1440A were used to acquire whole cell signals. Signals were sampled at 10 kHz and filtered at 2 kHz. AMPA receptor-mediated mEPSCs were collected at least 5 min after forming a stable whole cell patch clamping, recordings were performed by holding the MSN at -70 mV and perfused with aCSF supplied with 50 μ M DL- AP5 (Abcam), picrotoxin (100 μ M) (Tocris) and 1 μ M TTX (Tocris). The mEPSCs data were analyzed with Mini Analysis program (Synaptosoft). Recording of AMPA/NMDA current ratio were performed and data were analyzed as described previously (Saal et al., 2003). A platinum iridium concentric bipolar electrode (FHC) was positioned on the inner border of the corpus callosum between the cortex and dorsolateral striatum to stimulate the predominant cortical input to dorsolateral striatal region. After forming a stable whole cell recording for at least 5 min, stimulus intensity was adjusted to a value that could evoke a response between 200 to 300 pA when holding the MSN at -70 mV. After switching the holding potential to +40 mV, recordings were firstly performed in the presence of picrotoxin (100 μ M) and glycine (1 μ M) followed by isolating AMPA receptor mediated current through perfusion with DL- AP5 (50 μ M) additionally. Each evoked response was repeated for 10 times with an inter-stimulus interval of 20 s. To calculate the AMPAR/NMDAR current ratio, total AMPA plus NMDA component EPSC was obtained by averaging 10 consecutive responses in the absence of DL- AP5, pure AMPAR EPSC was then obtained by averaging 10 consecutive responses 5 min after application of DL- AP5. The average response in the presence of DL- AP5 was subtracted from the total component to obtain

an average NMDAR EPSC through Clampfit software. The peak of the AMPAR EPSC was divided by the peak of the NMDAR EPSC to generate an AMPAR/NMDAR current ratio. Series resistance was monitored online to ensure less than 25% changing throughout the experiment.

SPM and PSD preparation from mouse brain

Striatal and cortical tissue was obtained from two months old mice, experiments were performed as described previously (Peca et al., 2011). Briefly, mice were decapitated after an isofluorane overdose and the head was shock-frozen in liquid nitrogen for four seconds. Cortical (bregma 1.4 to -0.46 mm) and striatal (bregma 1.4 to -0.46 mm) regions were micro-dissected and snap-frozen on dry ice. Tissue from two mice for cortex samples or three mice for striatum samples was pooled to generate one sample (one n, approximately 200 mg brain tissue total). All the buffers and solution used for the SPM and PSD preparation were supplied with protease inhibitor (PI, cOmplete protease inhibitors from Roche). The pooled tissue was homogenized in 3 ml ice-cold buffer (4 mM HEPES pH 7.4, 0.32 M sucrose) using a 5 ml, tissue grinder (Wheaton# 358005) and power homogenizer at 900 rpm for 30-40 strokes. Homogenates were centrifuged for 15 min at 900 g at 4 °C. Supernatants were centrifuged again for 15 min at 900 g at 4 °C. Next, supernatants were centrifuged for 15 min at 18,000 g at 4 °C to obtain the crude synaptosomal fraction. This pellet was washed with 3 ml ice-cold buffer (4 mM HEPES pH 7.4, 0.32 M sucrose with PI) and centrifuged for 15 min at 18,000 g at 4 °C. The washed pellet was dissolved in 3 ml hypo-osmotic buffer (4 mM HEPES pH 7.4). Using the tissue grinder, 8 strokes were manually applied. Then the hypo-osmotic synaptosomal fraction was rotated for 1 h at 4 °C. Hypo-osmotic synaptosomal fractions were centrifuged for 20 min at 26,500 g at 4 °C and the pellets were snap frozen on dry ice. The pellets were supplemented with 300-400 µl buffer (50 mM HEPES pH 7.4,

2 mM EDTA) and dissolved via sonication (4°C, 10 % power, 30 % ON, 70 % OFF, 30 s, Omni-Ruptor 250). Using the bicinchoninic acid protein assay (Thermo Scientific Pierce), protein concentrations were measured for each sample. To prepare the PSD fraction, pellets of synaptosomal fractions were dissolved in 0.1 ml ice-cold buffer (4 mM HEPES pH 7.4, 0.32 M sucrose) and placed on top of the sucrose gradient 0.8 M/1.0 M/1.2 M (27%, 34%, 41%) in a Beckman ultracentrifuge tube (347356) with 500 ul/layer. Purification was performed by ultracentrifugation use swing bucket rotor TLS-55 at 117,257 g (42,000 rpm) for 2 hours at 4 °C. Enriched PSD fraction was harvested using 1 ml insulin-syringe with needle to punch a hole on the side around the interface between layer of 1.0 M and 1.2 M. About 400-600 ul of fraction was extracted and diluted to 0.32 M sucrose by adding 2.5x volume of HEPES buffer. Dissolved pellets were further spinned at 128,405 g for 30 min (55,000 rpm in TLA100.3). Pellet was resuspended after centrifuge with 1 ml of HEPES buffer (50 mM HEPES pH 7.4, 2 mM EDTA) followed by adding Triton X-100 to 0.5% (52.6 ul Triton X-100 10%). Mix thoroughly and rotate at 4 °C for 15 min then spin at 32.000g for 20 min (28.000rpm with TLA-100.3 rotor). And 1 ml ice-cold HEPES-C/ea into the pellet (PSD-1T), then add 0.5% Triton X-100 (52.6 ul Triton X-100 10%). Mix thoroughly and rotate at 4 °C for 15 min. Centrifuge again at 200.000g (65.000 rpm TLA-100.3) for 20 min to obtain PSD-2T pellet. Recover PSD-2T in 100 ul of HEPES buffer (50 mM HEPES pH 7.4, 2 mM EDTA). Add 14.24 ul 20% SDS and 43.94 ul 9M Urea. Brief sonication with 10% power and 3 pulses (30% ON-70% OFF) to dissolve the pellets. Aliquot the PSD fraction and store at -80 °C before protein quantification and western blotting assays.

Western blotting and quantification

The dissolved samples were mixed with 400 µl 2x Laemmli sample buffer (Bio-Rad). 400 µl of the mixture were kept without boiling and 400 µl were boiled for 5 min at 95 °C. Sample volumes corresponding to 25 µg protein amount per lane were loaded onto 4-15 % gradient Mini-PROTEAN TGX gels (Bio-Rad) and ran for 3 h at 50 V. The proteins were then transferred onto Whatman Protran nitrocellulose membranes (0.2 µm pore size, BA83, Sigma-Aldrich) using a tank blot system (Mini Trans-Blot Cell, Bio-Rad) for 120 min at 100 V at 4 °C. The membranes were blocked for 1 h with the respective blocking buffer (supplementary table 1) that did not contain any Tween-20. Subsequently, the membranes were incubated with primary antibodies diluted in blocking buffer (supplementary table 1) for 12 hrs at 4 °C. Following primary antibody incubation, the membranes were washed three times for 5 min per wash using TBST buffer (0.05 % Tween-20). Then, the secondary antibodies goat-anti-mouse IRDye680 (Li-COR Biosciences), donkey-anti-rabbit IRDye 800CW (Li-COR Biosciences) or goat-anti-rat IRDye 800CW (Li-COR Biosciences) diluted in 1:1 TBST (0.05 % Tween-20): Odyssey Blocking Buffer (Li-COR Biosciences) were incubated with the membrane for 2 hrs at room temperature. Following three rounds of washing with TBST, the membranes were scanned using an Odyssey CLx infrared imaging system (Li-COR Biosciences). Specific bands were then quantified with the contrast-independent, automatic background subtraction rectangular ROI tool of the built-in Software Image Studio 3.1 (Li-COR Biosciences) and normalized to an alpha-tubulin loading control for each lane and each blot. The values obtained for the mutation genotypes were then normalized to the wild-type expression. Statistical significance was tested for using the one-sample t-test for normalized values by asking whether the measured value differs significantly from the hypothetical value 1.0 (wild-type levels).

Golgi staining and spine counting

Golgi staining and spine counting was performed as described previously (Schmeisser et al., 2012). Three littermate pairs of male mice at two-month old from both R1117X and InsG3680 cohort were used for spine counting. Golgi staining of mouse brain was carried out according to the standard user manual (FD Rapid GolgiStain™ Kit). Briefly, dissected adult mouse brains were firstly immersed into impregnation solution (solution A+B) for 14 days in the dark, followed by incubating in solution C for 3 day before slicing. To prepare slices for imaging, coronal slices were prepared at 100 μ m thickness using vibratome. Z-stack confocal images were taken using an Olympus FluoView 1000 laser scanning confocal microscope. To quantify the spine density, images of at least ten neurons at the layer II/III of frontal association area for each mouse brain were taken with only one branch per neuron. Slices were imaged and the density of spines were counted by an experimenter blind to the genotype of the mice.

RNA isolation and Q-PCR assay

Tissue was obtained from age-and gender-matched brains of wild type, R1117X^{+/+} and InsG3680^{+/+} mice. Briefly, adult mice were decapitated after an isofluorane overdose and the head was shock-frozen in liquid nitrogen for four seconds. Cortical (bregma 1.4 to -0.46 mm) and striatal (bregma 1.4 to -0.46 mm) regions were micro-dissected and snap-frozen on dry ice. Quantitative PCR was performed as described previously (Wang et al., 2014). Total RNA was extracted using the RNeasy mini kit (QIAGEN) following the standard user manual. Equivalent amount of total mRNAs were reversely transcribed to cDNAs with iScript cDNA Synthesis Kit (Bio-Rad). Quantitative real-time PCR (q-PCR) was carried out using the iQ5 real-time PCR detection system

(Bio-Rad) with the iQ SYBR Green Super mix kit (Bio-Rad) following the guidance of manufacturer's manual. The primers used in this study are listed below:

Shank3 Exon 1 RT forward: 5'-CCGGACCTGCAACAAACGA-3'

Shank3 Exon 2 RT reverse: 5'-GCGCGTCTTGAAGGCTATGAT-3'

Shank3 Exon 6 RT forward: 5'-GTTGCGAGCTGCTTCTCCAT-3'

Shank3 Exon 8 RT reverse: 5'-GCGCAACTCTCCTGGTTGTA-3'

Shank3 Exon 16 RT forward: 5'-GGTTGGACACAAGCAAGTGG-3'

Shank3 Exon 17 RT reverse: 5'-CAGCCGTCATGGACTTGGAC-3'

Shank3 Exon 21 RT forward: 5'-CGGAAGCTTTGCACGAGAAC-3'

Shank3 Exon 21 RT reverse: 5'-CTCATCAATGGAGCGGGAGG-3'

Shank1 RT forward: 5'-CCGCTACAAGACCCGAGTCTA-3'

Shank1 RT reverse: 5'-CCTGAATCTGAGTCGTGGTAGTT-3'

Shank2 RT forward: 5'-AGAGGCCCCAGCTTATTCCAA-3'

Shank2 RT reverse: 5'-CAGGGGTATAGCTTCCAAGGC-3'

Gapdh RT forward: 5'-AAATGGTGAAGGTCGGTGTG-3'

Gapdh RT reverse: 5'-GCATTGCTGACAATCTTGAG-3'

Data Analysis

All comparisons between groups were collected from littermate animals with experiments performed at the same time. All results were presented as mean \pm SEM and were analyzed statistically using Student's *t*-test, one-way or two-way analysis of variance with proper post-hoc test as specified in legend of each figure (GraphPad Prism). * indicates $P < 0.05$; ** indicates $P < 0.01$; *** indicates $P < 0.001$.